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NORMAL HEMATOLOGICAL, BIOCHEMICAL, AND SERUM ELECTROLYTE VALUES FOR A COLONY OF RHESUS MONKEYS (*Macaca mulatta*)

Lieutenant Colonel James L. Kupper, USAF VC,
Captain Matthew J. Kessler, USAF VC, and Larry L. Cook

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SUMMARY PAGE*

THE PROBLEM

To establish normal hematological, biochemical, and serum electrolyte values for rhesus monkeys housed at the Naval Aerospace Medical Research Laboratory.

FINDINGS

Venipuncture was performed on 56 male and 57 female rhesus monkeys (Macaca mulatta) according to a standardized protocol to determine normal hematological, biochemical, and serum electrolyte values. Arithmetic means and standard deviations are tabulated for each parameter for each sex. The results from previously reported studies on rhesus monkeys are also tabulated and, where possible, values among the studies are compared.

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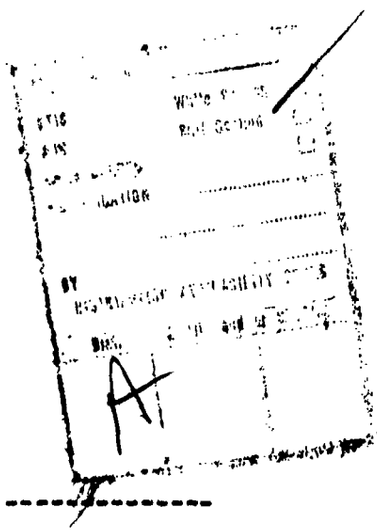

*The animals used in this study were handled in accordance with the Principles of Laboratory Animal Care established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science-National Research Council.

INTRODUCTION

A number of reports have been published in the scientific literature which detail the normal hematological, biochemical, and serum electrolyte values for captive rhesus monkeys (1-6, 8-22, 24-28). Information available from those studies was found to be of limited value for our purposes for various reasons. Sampling techniques, the use of pharmacological agents, handling procedures, and variations in laboratory methods and equipment all contributed to the differences reported among normal data and the results routinely obtained at the Naval Aerospace Medical Research Laboratory.

The specific purpose of this report is to establish normal values for our colony that were obtained under controlled and reproducible conditions to aid in the selection of monkeys for use in future experimentation.

SUBJECTS AND PROCEDURE

The subjects involved were rhesus monkeys purchased as a single shipment of 120 animals from another military research laboratory. The monkeys were maintained for 10 months prior to the study on a commercial laboratory primate chow* that was supplemented regularly with fresh apples and oranges. The sampled population consisted of 56 and 57 female monkeys whose ages were determined by dentition (7). The males ranged in age from 24 months to 54 months, with an arithmetic mean of 42.2 months and a standard deviation of 5.8 months. Female monkeys ranged from 24 months to 80 months, with an arithmetic mean of 50.1 months and a standard deviation of 8.8 months.

The male monkeys ranged in weight from 3.38 to 8.45 kilograms, with an arithmetic mean of 5.01 kilograms and a standard deviation of 1.2 kilograms. The females ranged in weight from 3.13 kilograms to 6.99 kilograms, with an arithmetic mean of 4.67 kilograms and a standard deviation of 0.74 kilograms.

The monkeys all received a comprehensive clinical physical examination. Of the 120 animals maintained in the colony, 113 monkeys were found to be healthy and in good physical condition prior to sampling. Among the seven monkeys not available for testing were those with major disabilities (amputated limbs) or severe illnesses (salmonellosis), and those that had died prior to the sampling date.

The subjects were deprived of all food for 17 hours prior to venipuncture, but were allowed water ad lib. Thirty minutes prior to

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*Purina Monkey Chow®, Ralston Purina Co., St. Louis, Missouri

venipuncture, the monkeys were administered a neuroleptic-narcotic combination drug, fentanyl-droperidol*, at a dosage of 1 cc per 40 pounds of body weight (1 cc per 18.2 kilograms of body weight) intramuscularly (23). This dosage regimen was sufficient to produce moderate catalepsis in the subjects and facilitate venipuncture. The animals were restrained in a horizontal position. The vast majority of blood samples were obtained from the femoral veins but, occasionally, samples were inadvertently obtained from the femoral arteries. Ten cubic centimeters of blood were collected using dry, sterile, disposable, 12-cc plastic syringes and 20-guage, 1-inch disposable needles. For routine blood counts the needle was removed from the tip of the syringe and 1 cc of blood was transferred without delay into a glass test tube that contained 1 drop of heparin at a concentration of 10,000 I.U. per cubic centimeter. The remaining 9 cc of whole blood were transferred from the syringe into glass test tubes that contained no anticoagulant. Blood smears were made from a drop of the heparinized blood sample.

METHODS

1. Hematology - Total erthrocyte count, total white blood cell count, and hematocrit were determined by using the Model ZBI Coulter counter. The counter was equipped with a channelyzer which displayed the cell-size distributions for accurate setting of the upper and lower window of the counter. Hemoglobin concentration was determined using a Coulter Electronics, Inc., hemoglobinometer. The procedure involved was the cyanomethemoglobin-coloimetric method. Mean corpuscular value (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from data obtained from the Coulter counter and Coulter hemoglobinometer. Differential white blood cell counts were performed by counting 100 white blood cells. An experienced hematology technician familar with rhesus monkeys performed the hematology studies.

2. Biochemistry - The Gilford Model 3500 automated chemistry analyzer was used for the measurement of serum chemistries in this study. Prepared reagents were obtained from Worthington Biochemical Corportation for the following chemistries: total protein, albumin, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, lactate dehydrogenase, creatinine phosphokinase, glucose, and triglycerides. Abbott Laboratories supplied the prepared reagents for blood urea nitrogen and cholesterol measurements. Serum lipoproteins and proteins were determined electrophoretically.

a. Total serum protein - The biuret reaction was employed in which cupric ions react with proteins in alkaline solution to form a blue-violet colored complex. A total protein standard of known protein concentration was used as a reference in the Gilford 3500.

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*Innovar , McNeil Laboratories, Fort Washington, PA

b. Serum albumin - Albumin reacts with the anionic bromcresol green dye and causes an increase in the green color. This color is measured at 628 nm and is directly proportional to the albumin concentration as compared to a serum albumin standard.

c. Serum glutamic oxaloacetic transaminase (SGOT) - The activity of SGOT is determined by coupling the reaction it catalyzes to a secondary reaction. In this secondary reaction NADH is oxidized to NAD, permitting the transaminase activity to be determined by the rate of decrease in absorbence at 340 nm.

d. Serum glutamic pyruvic transaminase (SGPT) - The activity of SGPT is proportional to the rate of a secondary reaction in which NADH is oxidized to NAO. This rate is measured spectrophotometrically at 340 nm.

e. Lactate dehydrogenase (LDH) - Lactate dehydrogenase catalyzes the conversion of lactate to pyruvate and NAD to NADH. LDH activity is directly proportional to the increase in absorbence of NADH measured at 340 nm.

f. Creatine phosphokinase (CPK) - CPK catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. The ATP produced and glucose are converted to ADP and glucose-6-phosphate by hexokinase in a secondary reaction. In a tertiary reaction catalyzed by glucose-6-phosphate dehydrogenase, NAD is reduced to NADH. The rate of NADH production measured at 340 nm is proportional to the CPK activity.

g. Serum glucose - In the primary reaction hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate. Glucose-6-phosphate is oxidized and NAD reduced in the secondary reaction by glucose-6-phosphate dehydrogenase. Since both reactions are essentially irreversible, the concentration of NADH produced in the secondary reaction, measured at 340 nm, is a direct measurement of the total glucose present in the sample serum when compared to a glucose standard.

h. Triglycerides - Serum triglycerides are first saponified with ethanolic potassium hydroxide to produce glycerol and free fatty acids. The free fatty acids are precipitated with magnesium sulfate. The glycerol is then measured by three coupled enzymatic reactions catalyzed by glycerol kinase, phosphokinase, and lactate dehydrogenase. In the third reaction NADH is oxidized to NAD. The total NADH decrease, measured at 340 nm, is directly proportional to the concentration of glycerol present after the saponification process and, therefore, to the concentration of triglycerides present in the sample.

i. Blood urea nitrogen (BUN) - Urease splits urea into ammonia and carbon dioxide. Glutamic dehydrogenase catalyzes the combination of the ammonia quantitatively with alpha-ketoglutarate along with the oxidation of NADH to NAD. The resultant decrease in absorbence at 340 nm is directly proportional to the concentration of ammonia formed which,

in turn, is quantitatively related to the concentration of urea initially present. This procedure is based on the comparison of the unknown samples to BUN standards.

j. Cholesterol - Cholesterol esters in serum are hydrolyzed to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a quinoneimine dye with an absorption maximum at 500 nm. The amount of color produced is directly proportional to the total cholesterol concentration of the sample as compared to cholesterol standards.

k. Lipoprotein - Lipoproteins were determined by electrophoresis on Gelman Sephrophore III cellulose acetate membranes, using a Beckman Microzone Cell. The membranes were stained using the Kohn ozonization method and measured with a Beckman Model CDS-100 computing densitometer.

l. Protein electrophoresis - The process was the same as that described for lipoprotein electrophoresis except that oil Red O was used for staining the Gelman Sephrophore III membranes.

3. Serum Electrolytes

a. Calcium - A manual fluorescent titration method was used to determine serum calcium concentration. The dye calcein fluoresces in the presence of calcium. This fluorescent complex is titrated with ethylenediamine tetra-acetic acid (EDTA), the end point being the termination of fluorescence as observed under a longwave ultraviolet light. Reagents were obtained from Oxford Laboratories.

b. Sodium and potassium - These two electrolytes were determined by using the International Laboratory Flame Photometer Model 153.

RESULTS AND DISCUSSION

Table I presents a summary of hematological values obtained from 56 male and 57 female monkeys sedated with Innovar®. Arithmetic means and standard deviations are tabulated for each sex group in Tables I-III.

The results of the biochemical serum analyses from the 113 sedated monkeys in the sampled population are listed in Table II, and the serum electrolyte values obtained from the same sample population of sedated monkeys are detailed in Table III.

Tables IV, V, and VI summarize the mean values of hematological, biochemical, and electrolyte values available in the literature on rhesus monkeys. Some authors reported results by male and female populations of monkeys, while others only reported values for both sexes combined. This resulted in some apparent discrepancies in Tables III, IV, and V. For instance, in Table IV, MCH determination, the range of means reported for combined sexes is greater than that for either sex group reported

separately. References are cited for each determination listed.

In general, the results accumulated from our subjects were consistent with previous findings reported in the literature. Notable exceptions include the following:

1. Hemoglobin values in grams percent were higher for the monkeys examined in the present study than in any other article reviewed. There is no obvious explanation for this inconsistency.

2. The female monkey mean corpuscular hemoglobin (MCH) in picograms was higher than all other reported values except for those of Robinson and Ziegler (19). The value reported for the male monkeys in our study exceeded all previous values reported in the literature.

3. The mean total white blood cell counts per cubic millimeter for our monkeys were generally lower than the means reported in most of the studies reviewed. The mean value of band neutrophils was higher than any of the other reported values. This is probably due to different interpretations of band cell types among hematology technicians.

4. The mean cholesterol values observed in our colony were lower than most values reported in the literature reviewed.

5. Potassium levels in millequivalents per liter for our female monkeys were below the normal range of reported mean values.

Normal mean values for lipoproteins (alpha, pre-beta, and beta), albumin, and globulins (alpha, beta, gamma) as a percentage of the total serum protein were not readily available in the literature. One study (22) reported serum lipoprotein profiles of twelve different primate species, but we were unable to compare those results with our values due to differences in analytical procedures and reporting units. Values for the normal mean triglyceride level in rhesus monkeys were also unavailable in published reports.

Values for normal serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, lactate dehydrogenase, and creatine phosphokinase expressed in international units per liter were not found in other reports. Since these determinations are method specific, they cannot be compared with values obtained by using different procedures. A review of these parameters expressed in various units can be found in Bourne (4).

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TABLE I

Hematological Values for the NAMRL Rhesus Monkey Colony

	Males(N=56)		Females(N=57)	
	Mean	S.D.	Mean	S.D.
erythrocytes x $10^6/mm^3$	5.25	0.46	5.00	0.39
hemoglobin gm%	15.1	1.1	14.6	1.0
hematocrit %	42.9	4.4	41.8	4.0
MCV microns^3	79.6	5.6	80.4	5.7
MCH picograms	29.4	1.1	26.7	1.4
MCHC %	38.3	2.4	32.8	1.8
leukocytes x $10^3/mm^3$	7.6	5.2	8.3	5.7
lymphocytes/100 WBC	43.8	9.6	57.3	9.5
neutrophils/100 WBC	32.0	8.4	35.2	8.6
band cells/100 WBC	1.8	1.4	1.9	1.0
monocytes/100 WBC	0.56	0.79	0.59	0.87
eosinophils/100 WBC	2.3	2.2	2.2	1.8
basophils/100 WBC	0.49	0.73	0.39	0.71

TABLE II

Biochemical Values for the NAMRL Rhesus Monkey Colony

	Males(N=56) Mean	S.D.	Females(N-57) Mean	S.D.
total protein gm%	7.4	0.58	7.6	0.71
albumin gm%	4.2	0.38	4.0	0.40
cholesterol mg%	146.9	28.5	140.9	25.0
BUN mg%	23.5	4.4	22.8	4.7
glucose mg%	102.4	20.1	89.8	18.3
triglyceride mg%	57.7	19.8	56.2	12.1
SGOT I.U./liter	28.4	9.0	28.1	8.1
SGPT I.U./liter	25.9	8.0	24.4	8.7
LDH I.U./liter	348.1	214.7	364.0	177.3
CPK I.U./liter	159.8	133.3	164.7	95.0
albumin % total protein	61.5	4.7	60.5	6.2
globulins % total protein				
alpha	8.8	2.0	9.2	2.6
beta	17.9	3.3	17.0	3.7
gamma	11.0	4.4	13.3	5.2
lipoproteins % total lipo-proteins				
alpha	64.1	3.0	64.3	1.0
pre-beta	10.6	6.9	10.1	7.7
beta	26.4	7.5	26.6	7.4

TABLE III

Serum Electrolyte Values for the NAMRL Rhesus Monkey Colony

mEq/L	Male(N=56) Mean	S.D.	Female(N=57) Mean	S.D.
calcium meq/liter	5.4	0.21	5.3	0.22
sodium meq/liter	147.2	3.8	147.5	5.1
potassium meq/liter	3.6	0.34	3.3	0.25

TABLE IV

Summary of Reported Mean Hematological Values for Rhesus Monkeys

Determination	Combined Sexes	Males	Females	References
erythrocytes x $10^6/mm^3$	4.32-6.38	5.54-5.64	5.10-5.67	3,4,8-12,15,19,21,24,26-28
hemoglobin gm%	11.7-13.5	13.2-13.3	12.3-13.4	3-5,8-12,15,19-21,24,27,28
hematocrit %	36.0-45.5	39.3-41.3	38.7-40.9	3-5,8,11,12,14,15,19,20,21, 24,27,28
MCV microns^3	69.9-91.5	69.9-74.8	70.2-76.3	3,4,8,15,19,21,28
MCH picograms	22.0-28.1	23.7	23.7-24.3	3,4,8,15,19,21,28
MCHC %	28.9-35.1	32.0-34.4	31.9-34.2	3,4,8,15,19,21,28
leukocytes x $10^3/mm^3$	7.8-16.9	8.2-10.6	8.9-12.2	3-5,8-12,14,15,19-21,24,26-28
lymphocytes/100 WBC	45.3-72.8	56.3-59.6	55.1-57.9	4,5,8-12,14,15,19,21,26-28
neutrophils/100 WBC	22.4-49.5	35.6-37.6	36.9-41.0	4,5,8-12,14,15,19,21,26-28
bands/100 WBC	0-0.4	0	0	4,5,8-12,14,15,19,21,26-28
monocytes/100 WBC	0-3.9	0-2.2	0-2.0	4,5,8-12,14,15,19,21,26-28
eosinophils/100 WBC	0-5.2	0-2.3	0-1.7	4,5,8-12,14,15,19,21,26-28
basophils/100 WBC	0-0.5	0-0.3	0-0.1	4,5,8-12,14,15,19,21,26-28

TABLE V

Summary of Reported Mean Biochemical Values for Rhesus Monkeys

Determination	Both Sexes	Males	Females	References
Total protein gm%	6.3-8.0	7.1-8.0	7.4-8.0	1,2,4,13,16,19,20,25,27
albumin gm%	3.6-5.0	4.2-5.0	4.4-4.7	1,2,4,6,13,19,20,25
cholesterol mg%	128-219	176	182	1,4,16.18,25,27
blood urea nitrogen mg%	10.1-25.3	10.1-22.2	12.8-22.9	1,4,16,18,20,25,27
glucose mg%	61.8-102.0	95.0-102.0	96.5-101.2	1,4,16-18,25,27

TABLE VI

Summary of Reported Mean Electrolyte Values for Rhesus Monkeys

Determination	Both Sexes	Male	Female	References
calcium meq/liter	4.9-5.8	5.3	4.9	2,4,11,13,16,17,19,25
sodium meq/liter	139.1-153.0	155.1-157.3	154.0-156.1	1,2,4,6,13,17,19,25,27
potassium meq/liter	3.4-5.2	5.1-5.2	5.1	1,2,4,6,13,17,19,25,27

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